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ASSESSMENT OF CYTOGENOTOXICITY INDUCED BY LEAD ACETATE ON ROOT TIP CELLS OF ONION *(Allium cepa* L.)

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ABSTRACT

The aim of present study was to assess the cytotoxic and genotoxic potency of lead acetate using an *Allium* root tip bioassay. Root tips of *Allium cepa* L. were treated with different concentrations of lead acetate *viz*., 0.03, 0.06, and 0.09 mg mL^{-1} of lead acetate for 48 h. Mitotic index (MI), phase index (PI), and total chromosomal abnormalities (TA) were calculated. The effect of lead acetate was found to be dose-dependent. The frequency of MI ranged from 8.4 to 5.7%, PI of prophase from 4.2 to 3.4%, PI of metaphase from 1.14 to 1.11%, PI of anaphase from 1.8 to 0.6%, and PI of telophase from 1.1 to 0.4%. The total chromosomal abnormality (TA) ranged from 0.42 to 1.39%. The differences between the averages of all the studied properties were significantly affected by the heavy metal lead acetate concentration variant applied. Mitotic observation showed that increasing of concentration variants doses decrease mitotic index and increased chromosomal abnormality percentage. Various types of physiological and clastogenic (laggard, stickiness, abnormal metaphase, chromatin bridge, and delayed anaphase were observed in *Allium cepa* root tip cells. Moreover, the *A. cepa* root tip bioassay method gives critical data for evaluating an agent's action mechanisms and impacts on genetic material.

Keywords: Abnormalities, *Allium cepa*, genotoxicity, lead acetate, mitotic index

INTRODUCTION

Heavy metal contamination in biosphere has shown increase during past few decades (Jarup, 2003). Most heavy metals have the potential to induce mutation and are frequently referred as environmental mutagens, carcinogens and clastogens. Lead is a major environmental mutagen and carcinogens; and reportedly induces various mutagenic, carcinogenic, and clastogenic effect in both plant and animals (Tchounwou *et al.,* 2012). Lead has drawn the attention of scientific fraternity due to its toxic nature and widespread use in different commercial products and industries related to food containers, paints, batteries, cosmetics, etc. (Kumar *et al.,* 2015). The genotoxic effect of lead depends on its concentration and duration of exposure (Jaishankar *et al.,*2014). Hence, it is necessity to assess the cyto-genotoxicity induced by lead.

In different species of higher plants, *Allium cepa, A. sativum, Zea mays, Hordeum vulgare* and *Tradescantia* have been used to evaluate the chromosomal anomalies (Enan, 2009). But *A. cepa* (onion) is regarded as the most favourable plant for assessment of chromosomal anomalies, probably due to the presence of good chromosomal condition, such as large chromosome and

reduced number (2n = 16) (Leme *et al.,* 2009). The *A. cepa* root chromosomal anomalies assay is widely used to determine cyto-genotoxicity induced by heavy metals (Wijeyaratne, 2020). *A. cepa* test has also been used for screening and assessing the cytotoxicity and genotoxicity (Saxena *et al.,* 2010, Mesi *et al.*, 2014; Denath *et al*., 2016; Kumari *et al.,* 2019). Therefore, the present work was aimed to screen and assess the cytotoxicity and genotoxicity induced by lead on *A. cepa* (onion) tip root cells at different concentration variant of lead acetate.

MATERIALS AND METHODS

Lead acetate was mixed with distilled water and its genotoxic effect were investigated by *Allium* root tip bioassay (Leme *et al.,* 2009). Three concentrations of lead (II) acetate trihydrate 0.03, 0.06, and 0.09 mg mL^{-1} were prepared in distilled water in different beakers. Average-sized healthy, mature purple colour onion weighing 15–20 g were obtained from local market Bhagalpur (India). The outer dead scales of bulb were removed by scalpel without damaging the roots primordia to promote the growth of new roots. *Allium cepa* bulbs (5 each) were directly placed in different solutions of lead acetate and allowed to germinate at a room temperature for 48 h. The roots produced were protected from direct sunlight. After the completion of 48 h treatment duration, the bulbs were removed, washed in distilled water and roots cut and fixed in Farmer's fluid (glacial acetic acid and ethanol in 1:3 ratio) for 24 h and then preserved in 70% ethanol. For chromosomal screening the preserved root tips were transferred in test tube with acetocarmine solution and warmed gently for 8-10 min and subsequently processed for cytological study by acetocarmine stain (0.5%) squash technique (Firbas -2017). The squash preparation was examined under a compound microscope at 40X and Screening of total cells was done by image J cell counter software (Grishagin, 2014).

The cyto-genotoxic analysis consisted of assessing the mitotic index percentage (MI%), phase index percentage (PI%) and total abnormality percentage (TA%). The mitotic index (MI), phase index (PI) and total abnormality (TA) were calculated as per the methods of Kpancasakti *et al*., (2012), Smail (2020), Shrivstava *et al.* (2020) and Verma *et al.* (2020). Various formulae used to calculate them were:

$$\text{Mitotic index [MI (\%)]} = \frac{\text{Total No. of dividing cells}}{\text{Total No.of cells counted}} \times 100$$

$$\text{Total abnormality [TA (\%)]} = \frac{\text{Total No. of abnormal cells}}{\text{Total No.of cells counted}} \times 100$$

$$\text{Phase index [PI (\%)]} = \frac{\text{Total No. of cells in a particular phase}}{\text{Total No.of cells counted}} \times 100$$

The results for each variant of treatment in these experiments were represented as an average of five experiments, and the mean values and standard error were calculated using Microsoft excel 209.

RESULTS AND DISCUSSION

Effect of lead acetate on mitotic index

Lead acetate treatment to *Allium* sp. revealed dose- and duration-dependent decrease in the mitotic index i.e. as the concentration of lead acetate increased the MI percentage of *Allium cepa* decreased as compared to the control (Table 1; Fig. 1). Mitotic index was 11.6% in control while mitotic index in lead acetate treatments of 0.03, 0.06 and 0.09 mg mL^{-1} was 8.4, 6.2 and 5.7% respectively.

Table 1: Effect of lead acetate on mitotic index and genotoxic effect on root tip cells of *Allium cepa*

Concentration of lead acetate (mg mL^{-1})	Total No. of cells counted	Total No. of dividing cells	Total No. of non-dividing cells	Mean ± S.E
0.00 (Control)	698	81	617	11.60 ± 1.20
0.03	700	59	641	8.4 ± 1.04
0.06	683	43	640	6.2 ± 0.92
0.09	719	41	678	5.7 ± 0.86

Effect of lead acetate on total abnormality in chromosomes

Lead acetate treatment revealed significant concentration- & dose-duration dependent increase in the percentage of total abnormality percentage of *Allium*. Treatment of lead acetate showed that TA increased with increase in lead acetate concentration. The overall maximum TA was 1.39% at 0.09 mg mL^{-1} and minimum of 0.42% at 0.03 mg mL^{-1}) (Table 2; Fig. 2). The total chromosomal abnormality (TA) ranged from 0.42 to 1.39% on different concentrations of lead acetate.

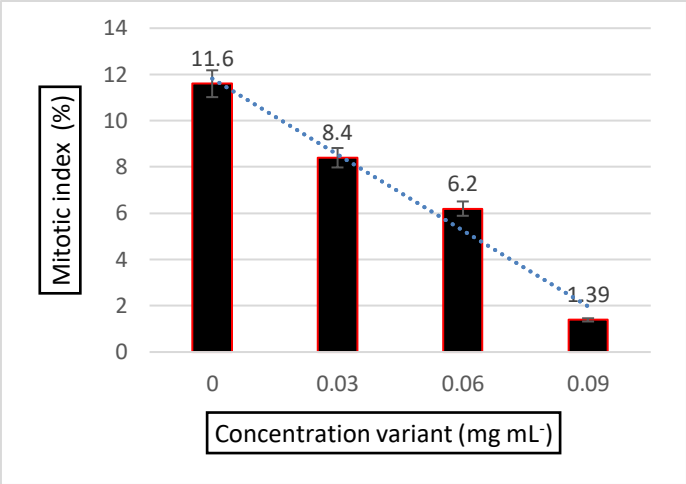


Fig. 1: Frequency of MI (%) at different concentration of lead acetate

Table 2: Status of phase index (%) of different stages of mitosis distribution and genotoxic effect of lead acetate (duration of treatment 48 h).

Conc. of lead acetate (mg mL^{-1})	Prophase		Metaphase		Anaphase		Telophase		TAP	
	No.	Mean ± SE	No.	Mean ± SE	No.	Mean ± SE	No.	Mean ± SE	No.	Mean ± SE
Control	42	6.0 ± 0.89	4	2.00 ± 0.52	15	2.1 ± 0.54	10	1.4 ± 0.44	0	0 ± 0
0.03	30	4.2 ± 0.75	8	1.14 ± 0.40	13	1.8 ± 0.50	08	1.1 ± 0.39	3	0.42 ± 0.24
0.06	26	3.8 ± 0.73	9	1.30 ± 0.43	04	0.5 ± 0.26	04	0.5 ± 0.26	8	1.17 ± 0.44
0.09	25	3.4 ± 0.67	8	1.11 ± 0.39	05	0.6 ± 0.28	03	0.4 ± 0.23	10	1.39 ± 0.43

* TAP – Total abnormalities percentage.

Effect of lead acetate on phase indices

Lead acetate treatment caused significant dose- and duration-dependent changes in the prophase index of *Allium*. Maximum percentage of phase index at was observed in control and minimum in higher concentration. Prophase index in control was 6.0% and at higher concentration 3.4%. (Table 2; Fig. 3). The metaphase index in control was 2.0% and at higher concentrations, Anaphase index in control was 2.1% and at higher concentration was 0.6%. Telophase index in control was 1.4% and at higher concentration 0.4%. In

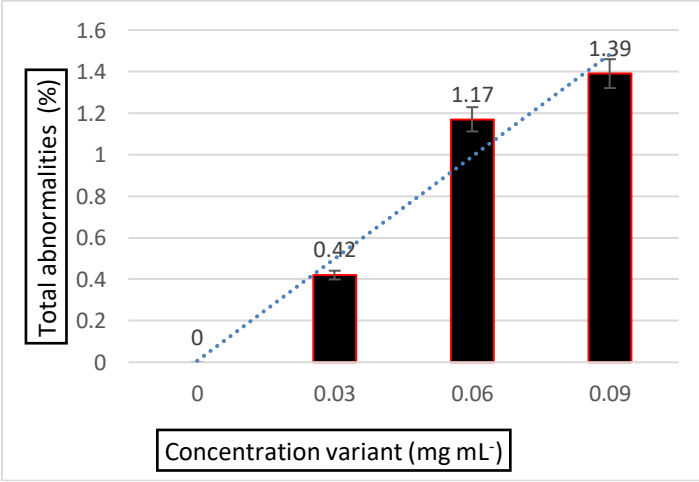


Fig. 2: Freqency of TA (%) at various concentrations of lead

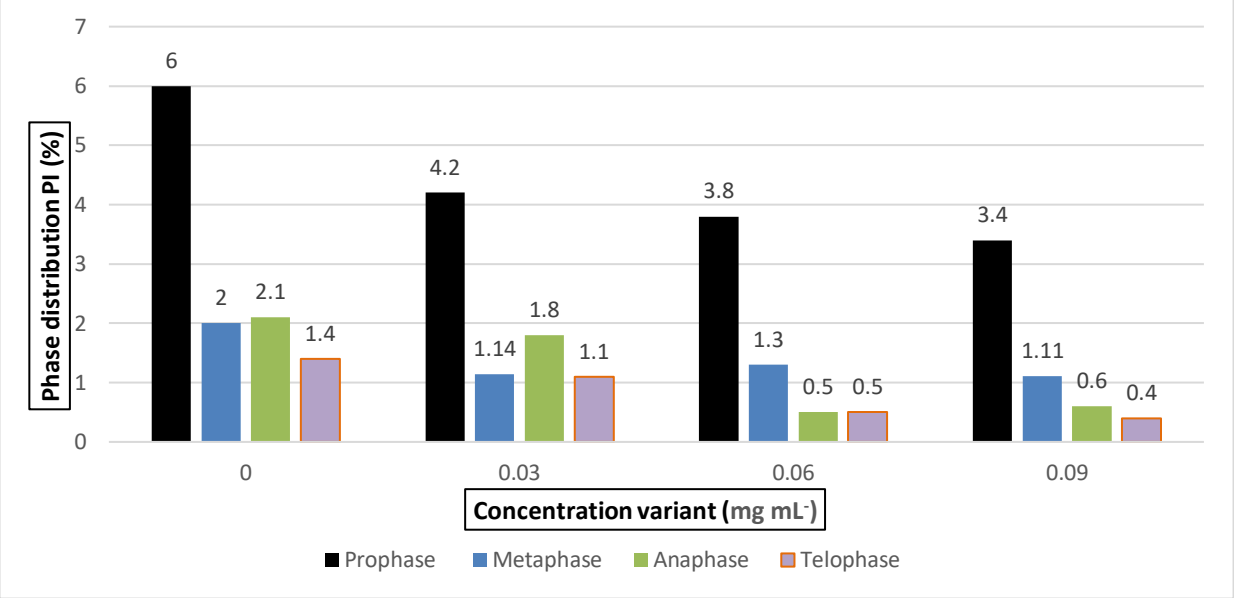
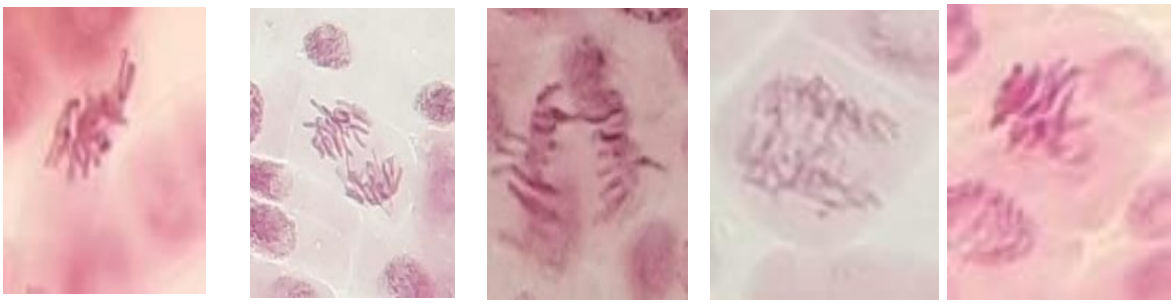


Fig. 3: Frequency of PI (%) at various concentrations of lead



A B C D E

Fig. 4: Effect of lead acetate on the root tip cells of onion (*Allium cepa*); A: Improper metaphase, B: Chromosome bridge, C: Laggard, D: Improper anaphase, E: Stickiness

present study the potential cytotoxic and genotoxic effects of lead acetate on *Allium cepa* root tip was observed as well as various types of physiological and clastogenic (laggard, stickiness, abnormal metaphase, chromatin bridge and delayed anaphase) were noticed in *Allium* root tip cells. These results are in agreement with Vazhangat *et al*. (2016) and Sabeen *et al*. (2020).

Conclusions: The present study provides information about the cytotoxic and genotoxic effects of lead acetate on mitotic index and changes in chromosomal abnormalities an *Allium cepa* root tip bioassay. The result revealed the health and environmental risks by heavy metal discharge into the water bodies and soil. The *Allium cepa* root tip bioassay may serve as an integral tool to evaluate and monitor the quality water bodies. Furthermore, the *Allium cepa* test method gives critical data for evaluating an agent's action mechanisms and impacts on genetic material (mutagenic and/or clastogenic effects). Despite all of the benefits that the *Allium cepa* test system provides, it has been frequently utilized to investigate the effects of xenobiotics, establishing itself as a major tool for environmental monitoring studies with positive findings.

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REFERENCES

Debnath, B., Paul C., Debnath, A. and Saha, D. 2016. Evaluation of cytotoxicity of *Terminalia arjuna* (Roxb.) Wight & Arn and *Moringa oleifera* Lam. in root meristem cells of *Allium cepa* L. *Journal of Medicinal Plants Studies*, **4**(3): 107-110.

Enan, M.R. 2009. Genotoxicity of the herbicide 2, 4-dichlorophenoxyacetic acid (2, 4-D): Higher plants as monitoring systems. *American-Eurasian Journal of Sustainable Agriculture*, **3**(3): 452-459.

Firbas, P. 2017. Genotoxicity testing with *Allium* M test daring the cleaning process for constructed wetland (CW) and environmental quality samples, Slovenia. *SF J Materials Research Letters*, **1**(2): 1-14.

Grishagin I. V. 2015. Automatic cell counting with ImageJ. *Analytical Biochemistry*, **473**: 63-65.

Jarup, L. 2003. Hazards of heavy metal contamination, *British Medical Bulletin*, **68**(1): 167-182.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B.B. and Beeregowda, K.N. 2014. Toxicity mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, **7**(2): 60-72.

Kpancasakti, H., Lunggani, A.T. and Nurhakim, D.M.A. 2012. Chromosome and mitotic cell division phase in onion roots after 24 hours acetoorcein soaking time. *BIOMA*, **14**(2): 46-48.

Kumar, V. and Nagpal, A.K. 2015. Genotoxic effect of lead acetate employing *Allium sativum* root chromosomal aberration assay. *International Journal of Advanced Research in Biological Sciences*, **2**(1): 193-199.

Kumari, R., Kumari, D. and Rani, A.K. 2019. Cytotoxic and genotoxic effect of profenos on root tip cells of *Allium cepa* (onion). *International Journal of Research and Analytical Reviews*, **6**(2): 676- 680.

Leme, D.M. and Marin-Morales, M.A. 2009. *Allium cepa* test in environmental monitoring: A review on its application. *Mutation Research*, **682**(1): 71-81.

Mesi, A.D. and Kopliku, D. 2014. Toxic potency of metal -doped river water (Cr, Cu and Pb), on *Allium cepa* L., An albanian case. *Athens Journal of Science*, **1**(4): 255-268.

Sabeen, M., Mahmood, Q., Bhatti Z.A., Faridullah, I.M., Bilal, M., Hayat, M.T., Irshad, U., Akbar, T.A., Arslan, M. and Shahid, N. 2020. *Allium cepa* assay based comparative study of selected vegetables and the chromosomal aberrations due to heavy metal accumulation. *Saudi Journal of Biological Sciences*, **27**(5): 1368-1374.

Saxena, P.N., Gupta, S.K. and Murthy, R.C. 2010. Carbofuran induced cytogenetics in root meristem cells of *Allium cepa* and *Allium sativum*: A spectroscopic approach for chromosome damage. *Pesticide Biochemistry and Physiology*, **96**(2): 93-100.

Shrivastava, G., Pagare, S., Khanna, A. and Aglawe, V. 2020. Antimitotic effect of artesunate and tulsi extract on onion root tip system. *Research Journal of Life Science, Bioinformatic, Pharmaceuticals and Chemical Sciences*, **6**(4): 35-46.

Smail, H.O. 2020. Impact of different concentration of sodium chloride on the root growth, cell division and chromosomal abnormalities in the root tips of *Allium cepa*. *Journal of Advanced Laboratory Research in Biology*, **11**(1): 1-6.

Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K. and Sutton, D.J. 2012. Heavy metal toxicity and the environment. *Experientia Supplementum*, **2012**(101): 133-164.

Vazhangat, P. and Thoppil, J.E. 2016. Apoptotic induction via membrane/DNA damage and metabolic inactivation by synthetic food colorants in *Allium cepa* root meristem. *Turkish Journal of Biology*, **2016** (40): 922-933.

Verma, N. and Kumari, D. 2020. Evaluation of the cytotoxic effect of paracetamol on onion root tip cell. *World Journal of Pharmaceutical Research*, **10**(2): 1184-1189.

Wijeyaratne, W. and Wickramasinghe, P. 2020. Chromosomal abnormalities in *Allium cepa* induced by treated textile effluents: Spatial and temporal variations. *Journal of Toxicology*, ***2020***: 1-10